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Commissioner for Patents  
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Presented for filing is a new original patent application of:

Applicant: Nozaki, et al.

Title: METHOD AND APPARATUS FOR DISPLAYING GENE  
EXPRESSION PATTERNS

Enclosed are the following papers, including those required to receive a filing date  
under 37 CFR §1.53(b):

	Pages
Specification	32
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Abstract	1
Declaration	4
Drawing(s)	26

Enclosures:

- Assignment cover sheet and an assignment, 3 pages, and a separate \$40 fee.
- Certified copies of priority document nos. 277918/1999 and 088695/2000.
- Postcard.

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Under 35 USC 119, this application claims the benefit of foreign priority applications filed in Japan, serial number 277918/1999, filed September 30, 2000 and serial number 88695/2000, filed March 28, 2000.

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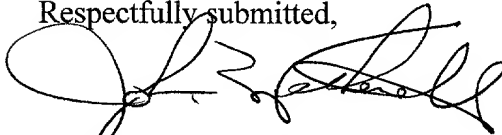
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Respectfully submitted,



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Enclosures  
JRW/rzm

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APPLICATION  
FOR  
UNITED STATES LETTERS PATENT

TITLE: METHOD AND APPARATUS FOR DISPLAYING GENE  
EXPRESSION PATTERNS

APPLICANT: Yasuyuki Nozaki, Ryo Nakashige, Tsunehiko Watanabe and  
Takuro Tamura

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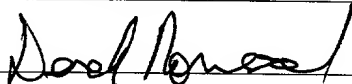
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units or function groups. The interactions between the function units or function groups are also analyzed by correlating with known enzymatic reaction data or metabolism data, or more directly, by knocking out or overreacting a specific gene to eliminate or accelerate expression of the gene to study the direct and indirect influences on the gene expression patterns of the whole collection of genes.

One successful case in this art field is the expression analysis of yeast by the group of P. Brown from the Stanford University (Michel B. Eisen et al., Clustering analysis and display of genome-wide expression patterns, *Proc. Natl. Acad. Sci.* (1998), Dec 8; 95(25): 14863-8). They hybridized genes with a gene extracted from a cell in a time series using a DNA microarray and numerated the expression levels thereof (i.e., numerated the brightness of the hybridized fluorescent signals). By converting the values into colors, the expression pattern of each gene can be displayed in a visually apprehensible manner. At this point, genes that have a similar expression pattern during their gene cycles (genes having closer expression levels at the same point) are clustered together.

Figure 24 is a diagram showing an example of displaying expression status 2400 of genes according to the above-described method, where the horizontal and vertical axes indicate time and genes, respectively. In this display, genes belonging to a common cluster may be considered to have common functional characteristics. In Figure 24, each of the blocks 2401 represents expression status of a gene at one time point. In the figure, the expression status is schematically represented in a gray scale format.

Figure 25 is a diagram showing an example of displaying

expression status 2500 of genes according to the above-described method, where the horizontal and vertical axes indicate experiment cases and genes, respectively. A dendrogram shown on the left is made by stepwisely joining every two most similar clusters together. The length of each branch corresponds to the distance between the two joined clusters. In Figure 25, each of the blocks 2501 represents an expression status of a gene at one time point. In the figure, the expression status is schematically represented in a gray scale format.

The above-described displaying method allows a supposition that genes belonging to the same cluster may possibly share common functional characteristics.

With the gene expression patterns, however, it is not so simple as to elucidate the relationship among all of the genes in a cell by finding some gene groups having similar expression patterns for the entire cell cycle.

For example, different genes may exhibit similar expression for having similar function at a certain time point. However, they may have different roles at other time point, at which point, of course, the expressions are different. According to the conventional method in which similar expression patterns are clustered together over the entire cell cycle, these genes are classified into different clusters. Therefore, it is difficult to find the above-mentioned characteristics.

In an actual analysis of gene expression patterns, enormous amount of data will be subjected to clustering as shown in Figure 25. The number of genes is several thousands to ten-thousands, or more than hundred-thousands at maximum. The experiment cases (data) employed may be of any number, for example, in an order of about ten



results of clustering so that a user can select a desirable level of the grouping for more comprehensible display to study the groupings of the genes. In other words, the present invention has an objective of providing a method and an apparatus for effectively displaying gene expression patterns by providing multiple selectable clustering levels.

#### SUMMARY OF THE INVENTION

In order to achieve the above-described objectives, the present invention provides a method for displaying gene expression pattern for visually displaying time sequential expression patterns of multiple genes whose expressions change according to experiment cases where a first axis represents the genes and a second axis represents the experiment cases,, the method comprising the steps of: designating a segment along the second axis in the expression pattern data of the multiple genes; and clustering the expression pattern data within the designated segment along the second axis based on a predetermined reference value, repeating clustering within the same cluster in a forward or reverse direction along the second axis while changing the reference value, and displaying the results according to a predetermined display format.

The reference value refers to value for determining whether expression patterns of distinct genes are the same or different.

Furthermore, the present invention displays two or more different genes according to the predetermined display format where they have the same expression pattern at the beginning but become to have different expression patterns within the segment along the second axis.

The present invention also displays two or more different

genes according to the predetermined display format where they have different expression patterns at the beginning but become to have the same expression pattern within the segment along the second axis.

The experiment cases may be time sequential experiments, states of individual's tissue, species of individuals, individual's sites, or presence and absence of an artificial condition. Alternatively, the experiment cases may be combinations of some of the group consisting of time sequential experiments, states of individual's tissue, species of individuals, individual's sites, and presence and absence of an artificial condition.

The present invention is also an apparatus for analyzing gene expression patterns, which acquires, from a database, expression pattern data of multiple genes whose expressions change according to experiment cases, and which visually displays the expression patterns on a screen of a display device where a first axis represents the genes and a second axis represents the experiment cases, the apparatus comprising:

an inputting means for designating a segment along the second axis in the expression pattern data of the multiple genes obtained from the database; and

an arithmetic unit for clustering the expression pattern data within the designated segment along the second axis based on a predetermined reference value, repeating clustering within the same cluster in a forward or reverse direction along the second axis while changing the reference value, and displaying the results according to a predetermined display format.

In order to achieve the above-described objectives, the present invention comprises a step of displaying a round number of the cluster groups for each clustering phase taking a recognition

error range in consideration, for the results of the clustering analysis of the gene expression pattern data.

For example, the results of the analysis at multiple phases are stored regardless of the recognition error range input by a user so that the user can choose the briefness of the analysis results to be displayed from multiple phases presented.

In displaying the results, a phase scale bar and a dendrogram cross-cutting line are provided for selecting the phase from the multiple phases. The user can select a specific result at a desirable phase by moving the dendrogram cross-cutting line on the phase scale bar. Upon moving the dendrogram cross-cutting line on the phase scale bar, the number of gene groups (clusters) at that phase. Moreover, dividing lines for distinguishing the gene groups at that phase are displayed, and the gene groups of a predetermined size are specified.

This specification includes part or all of the contents as disclosed in the specifications and/or drawings of Japanese Patent Applications Nos. 11-277918 and 2000-88695 which are priority documents of the present invention.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic view showing one embodiment of an analyzing apparatus according to the present invention.

Figure 2 is a schematic view showing one example of gene expression pattern where a narrowed region has been subjected to clustering in a small range. (in forward time direction)

Figure 3 is a schematic view showing another example of gene expression pattern where a narrowed region has been subjected to clustering in a small range. (in reverse time direction)

Figure 4 is a flowchart showing a scheme of the clustering process.

Figure 5 is a diagram for illustrating the relationship between the variables used for clustering and the actual data.

Figure 6 is a flowchart showing an algorithm with respect to the process of setting the initial parameters.

Figure 7 is a flowchart showing an algorithm of the process of determining the display area.

Figure 8 is a flowchart showing an algorithm for clustering.

Figure 9 is a flowchart showing the process following the process shown in Figure 8.

Figure 10 is a flowchart showing a general algorithm for displaying.

Figure 11 is a diagram for illustrating an exemplary display of gene expression patterns resulting from clustering carried out by shifting a slit from the beginning of the clustering-applied region in a forward direction along the time axis.

Figure 12 is a diagram for illustrating an exemplary display of gene expression patterns resulting from clustering carried out by shifting a slit from the end of the clustering-applied region in a reverse direction along the time axis.

Figure 13 is a schematic view showing an exemplary display of the results of a clustering analysis of gene expression patterns according to the present invention.

Figure 14 is a schematic diagram showing an exemplary structure of gene expression pattern data.

Figure 15 is a schematic diagram showing an exemplary structure of a cluster.

Figure 16 is a schematic diagram showing an example of generating a tree structure of clusters.

Figure 17 is a diagram showing an example of display data.

Figure 18 is a flowchart showing a general process of displaying gene expression patterns according to the invention.

Figure 19 is a flowchart for illustrating clustering analysis with respect to the process of generating a cluster tree.

Figure 20 is a flowchart for illustrating the clustering analysis with respect to the process of setting the cluster level.

Figure 21 is a flowchart for illustrating the clustering analysis with respect to the process of generating display data.

Figure 22 is a flowchart showing Process A (in Figure 21) of display data generation in detail.

Figure 23 is a view showing an example of combining and clustering cancerous and normal cells.

Figure 24 is a diagram for illustrating an exemplary display of gene expression patterns obtained by clustering similar expression patterns together over the entire cell process.

Figure 25 is a diagram showing an exemplary display of the results of a standard clustering analysis of gene expression patterns.

Figure 26 is a schematic diagram showing an exemplary display of the entire results of clustering analysis and a corresponding display of the targeted cluster tree.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

Hereinafter, the present invention will be described in more detail with reference to the accompanying drawings. In the following example, time sequential experiments are exemplified as typical experiment cases.

Figure 1 is a schematic view showing a system structure of one embodiment of an apparatus for analyzing gene expression patterns, which employs a method of the invention for displaying gene expression patterns. The analyzing apparatus of this embodiment is provided with a storage medium (or database) 101 for storing data of gene expression patterns obtained by numerating the levels of gene expressions obtained during a series of cell process, a display 102 for visualizing and displaying the data of expression patterns, a keyboard 103 and a mouse 104 for inputting values into the system or for selection, and a clustering processor 105 for clustering the expression pattern data along the course of gene expressions. The clustering processor 105 is embodied with a computer and a program therefor.

An alternative embodiment is configured such that the data of gene expression patterns is acquired from, instead of the storage medium 101, database supervised by a remotely provided server computer via a network or the like.

According to this embodiment, a time segment is designated among the cell cycle to perform clustering within this time segment in a small range.

Specifically, genes belonging to the same cluster are gathered together and a dividing line is drawn between distinctive clusters. Clustering is further performed for the genes belonging to the same cluster. As shown in Figure 2, when clustering is repeatedly performed in small range from the beginning of the

clustering-applied region in the forward direction along the time axis, the course of gene expressions can be expressed as a tree structure. In Figure 2, the designated time segment is shown as a clustering-applied region 201.

In other words, the expression patterns within the clustering-applied region are arranged that they have the same expression levels at the beginning but become different at some point in the time segment. When such a display is obtained, it can be assumed that different genes exhibited similar expressions at the beginning for having the same function, but became to give different expressions at some time point for having different roles.

Similarly, when small-range clustering is performed from the end of the clustering-applied region in reverse time direction, the gene expression patterns may be arranged into a reverse-tree structure as shown in Figure 3.

This display indicates expression patterns that are different at the beginning of the region but become the same at some point in the time segment. It can be assumed that, in this case, different genes have different functions at the beginning but at some time point become to have similar roles.

Figure 4 is a flowchart showing a scheme of algorithm employed in the clustering processor 105 for clustering and displaying gene expression pattern data.

First, initial parameters are set (Step 401) and a display area is determined (Step 402). The initial parameters will be described later in more detail. Then, displaying process is performed (Step 403), whereby the whole processing is ended. The present algorithm is for displaying expression patterns of different

genes which at the beginning are the same but becomes different at some time point (Figure 2).

Figure 5 is an illustration showing the relationship between the variables used in the present algorithm and the actual data. Figure 6 is a flowchart showing details of the algorithm with respect to the process of setting the initial parameters (Step 401 in Figure 4).

First, data of gene expression patterns are read out from the storage medium 101. As shown in Figure 5, the data of gene expression patterns contains expression pattern data of  $m+1$  number of sample genes  $g_0, g_1, \dots, g_m$ , obtained by experiments at time  $T_0, T_1, \dots, T_n$ . The observed expression value of gene  $g_i$  at time  $T_j$  is indicated as  $g[j][i]$  (Step 601).

Then, the keyboard 103 and the mouse 104 are used to input a clustering-applied region (starting time  $T_{start}$  and ending time  $T_{end}$ ), positive values  $(K_{start}, K_{start+1}, \dots, K_{end})$  as reference values for distinguishing clusters, an integer (S) indicating a range of clustering, and the methods of clustering (Step 602).

The solid lines 201 in Figures 2 and 3 indicate the clustering-applied regions, namely a time segment within the cell cycle designated for further detailed clustering. For example, if a cell shows a particular expression pattern at some time point during the cell cycle, the clustering-applied region may be designated to include that time point to monitor in further detail the expression status of every gene. The present invention is different from the conventional clustering fundamentally in that it does not cluster genes with similar expression status over the entire cell cycle as shown in Figure 24, but arranges the gene patterns such that different genes have the same expression patterns at the beginning

of the region but become to have different expression patterns within the region as shown in Figure 2.

The reference value for distinction between different clusters is a minimum value, namely threshold  $K$ , of dissimilarity between the clusters. Since thresholds can variably be set as  $K_{start}$ ,  $K_{start+1}, \dots, K_{end}$ , the level of clustering may be regulated to be brief or detailed along the time axis.

The present system does not target the entire expression data obtained at time  $T_0, T_1, \dots, T_n$  for calculating dissimilarity for clustering, but selects a certain time segment to target data within that time segment for calculating dissimilarity. As shown in Figure 5, this time segment is called a slit 501 and the length  $S$  (a width along the time axis) of this slit 501 is called a clustering range. According to the present algorithm, the beginning of the slit 501 is set to  $T_{start}$  for clustering data within a range  $T_{start}$  to  $T_{start+S}$ . Then, the slit 501 is shifted in a forward direction along the time axis to cluster each of the grouped clusters within a range  $T_{start+1}$  to  $T_{start+S+1}$ . This procedure is repeated until the end of the slit reaches  $T_{end}$ . The smaller the clustering range is (i.e., the shorter the width of the time segment is), the more detailed difference between the gene expressions will be obtained.

In the clustering process, the similarity or dissimilarity which indicates correlation between the genes upon clustering (Pearson's correlation coefficient, squared Euclidean distance, standardized squared Euclidean distance, Mahalanobis' distance, Minkowsky distance, etc.) and algorithm for joining the clusters (nearest neighbor method, furthest neighbor method, group average method, centroid method, median method, Ward method, flexible method, etc.) are designated. The present algorithm utilizes dissimilarity.

When similarity is selected for clustering, a minus sign may be applied in front of the calculated similarity to give an inverse number as for conversion to dissimilarity.

Once these values are set, each item is checked whether it is proper or not. The items are (i) whether the clustering-applied region  $T_{\text{start}}$  to  $T_{\text{end}}$  is included in the region  $T_0$  to  $T_n$  (Step 603), (ii) whether the clustering range  $S$  is within the width of the clustering-applied region ( $S \leq \text{end-start}$ ) (Step 604), and (iii) whether the similarity or dissimilarity complies with the algorithm of the selected clustering method (for example, when centroid method, median method or Ward method is employed as the algorithm, squared Euclidean distance should be selected) (Step 606). If any of these values is not proper, an error signal is output to the display device 102 to demand re-entering (Step 607).

When all of the set items are confirmed proper, an average level  $G_i = (g[0][i] + g[1][i] + \dots + g[n][i]) / n$  of expressions of a gene  $g_i$  (where  $i = 1, 2, \dots, m$ ) is calculated (Step 608).

In order to store display information of each gene, an array  $l[I]$  ( $I = 0, 1, \dots, m$ ) 502 (Figure 5) and an integer variable  $lmax$  are prepared. Each  $l[I]$  is a structure data that consists of a member indicating an index of a gene ( $index$ ) and a member indicating the location of the dividing line between distinctive clusters ( $linepos$ ) as shown in Figure 5. The members of the structure can be set or referred to as  $l[I].index$  or  $l[I].linepos$ . For all "I", the value of  $l[I].linepos$  is initiated as  $T_{\text{end}}$  (Step 609), and the value of  $lmax$  is set to "0" (Step 610). Then, the value of "start" is set to variable  $t$  (Step 611).

The present algorithm employs an abstract data type called "cluster" which indicates set of integers. A cluster has an

interface for registration and deletion of an integer and for reference of the registered data.

Finally, cluster B is generated to which  $\{0,1,2,\dots,m\}$  is registered (Step 612), whereby the whole process is ended.

After the above-described initialization, the clustering-applied region 201 is processed. Specifically, the display area is determined by using t and B set above as arguments (Process A in Step 402 of Figure 4).

Figure 7 is a flowchart showing in detail the process of determining the display area (Process A) in Figure 4. The display information is registered in the array 1 during this Process A.

First, let the given cluster and time be B and t, respectively, as arguments (Step 701). Then, B is subjected to clustering (Process B) where t and B are given as arguments. As a result of Process B, the total number of the clusters and the clustering results are set to cmax and A[J] ( $J=1,2,\dots,cmax$ ), respectively (Step 702). Details of Process B will be described later.

Then, whether "t + S" equals "end" is judged (Step 703). When they are equal, it means that the end of the slit 501 has reached the end of the clustering-applied region 201, whereby the clustering process is ended. The following process is executed for each cluster until J exceeds cmax (starting from  $J = 1$ ) (Steps 704 and 705). Where the elements of cluster A[J] are  $\{i_1,\dots,i_k\}$ , these elements are aligned and displayed under a certain rule. Here, the average of the expression levels  $G_{i1},\dots,G_{ik}$  corresponding to the elements are aligned in a descending order to be rearranged as  $G_{j1},\dots,G_{jk}$  (Step 706).

Then the value for array  $l$  is entered. Specifically, " $l[]$ .index" which indicates the positional information of the expression pattern data is set as  $l[lmax].index = j_1$ ,  $l[lmax+1].index = j_2, \dots, l[lmax+k-1].index = j_k$  such that they are arranged in a descending order according to their average brightness (Step 707). The value " $t$ " is entered into " $l[lmax+k-1].linepos$ " which indicates the line for dividing distinctive clusters (a solid line 202 extending in horizontal direction is representatively shown in Figure 2) from time " $t$ " to " $t + S (= T_{end})$ " (Step 708).

Next,  $k$  is added to  $lmax$  for indicating the maximum number of the already entered data of array  $l$  (Step 709). Then,  $J$  is incremented to perform the next clustering (Step 710).

On the other hand, when " $t + S$ " does not equal "end" (i.e., when the end of the slit 501 does not reach the end of the clustering-applied region 201) at Step 703,  $t$  is incremented and  $J$  is set to "1" (Step 711). The following procedure is performed for each cluster until  $J$  exceeds  $cmax$  (Step 712). Specifically,  $A[J]$  is substituted for  $B$  (Step 713), and the display area is determined using time  $t$  and cluster  $B$  as arguments (Process A) (Step 714). Then, the value " $t$ " is entered into " $l[lmax-1].linepos$ " which indicates a line for dividing distinctive clusters from time " $t$ " to " $T_{end}$ " (Step 715). Then,  $J$  is incremented to perform the next clustering (Step 716). If the procedure is finished for all clusters  $A[J]$  ( $J = 1, \dots, cmax$ ), the process is ended.

Figures 8 and 9 are flowcharts showing algorithm of the clustering process (Process B).

First, the entered cluster and time are used as arguments  $B$  and  $t$ , respectively (Step 801).

Then, where the elements of cluster B are  $i_1, \dots, i_k$ , similarity or dissimilarity  $d_{ij}$  ( $i < j$  and  $i, j \in \{i_1, i_2, \dots, i_k\}$ ) between genes corresponding to  $i_1, \dots, i_k$  from time  $t$  to time  $t + S$  is calculated (Step 802).

The similarity (dissimilarity) of gene expression data  $\{g[0][i], g[1][i], \dots, g[n][i]\}$  of genes  $g_i, g_j$  is a value obtained, for example, by the following calculation (Step 802).

(1) When Pearson's correlation coefficient is designated as similarity,

$$d_{i,j} = \frac{\sum_{k=t}^{t+S} (g[k][i] - \overline{g[i]})(g[k][j] - \overline{g[j]})}{\sqrt{\left\{ \sum_{k=t}^{t+S} (g[k][i] - \overline{g[i]})^2 \right\} \left\{ \sum_{k=t}^{t+S} (g[k][j] - \overline{g[j]})^2 \right\}}} \quad \dots\dots(1)$$

$$\text{where } \overline{g[l]} = \frac{1}{S} \sum_{k=t}^{t+S} g[k][l]$$

Since the present algorithm targets dissimilarity, the obtained similarity has to be converted to dissimilarity by providing with a minus sign to give an inverse number.

(2) When squared Euclidean distance is designated as dissimilarity,

$$d_{i,j} = \sum_{k=t}^{t+S} (g[k][i] - g[k][j])^2 \quad \dots\dots(2)$$

(3) When standardized squared Euclidean distance is designated,

$$d_{i,j} = \sum_{k=t}^{t+S} (g[k][i] - g[k][j])^2 / s_k^2 \quad \dots\dots(3)$$

where  $s_k^2$  is a variance of variable  $g[k][0], \dots, g[k][n]$

(4) When Mahalanobis' distance is designated,

$$d_{i,j} = (g[i] - g[j])S^{-1}(g[i] - g[j]) \quad \dots\dots(4)$$

where  $g[l] = (g[t][l], \dots, g[t+S][l])$ , and  $S$  is covariance matrix of  $g[i], g[j]$

(5) When Minkowsky distance is designated,

$$d_{i,j} = \left\{ \sum_{l=t}^{t+S} |g[l][i] - g[l][j]|^k \right\}^{1/k} \quad \dots\dots(5)$$

Then, clusters  $C[1], \dots, C[k]$  are generated which are registered as  $C[1] \leftarrow \{i_1\}, \dots, C[k] \leftarrow \{i_k\}$  (Step 803). Variable  $ccnt$  indicating the number of the generated clusters is substituted with  $k$  (Step 804). Then, a null set of cluster  $D$  is generated (Step 805).

Then the minimum value  $d_{p,q}$  of the calculated dissimilarity  $d_{i,j}$  ( $i, j \in \{1, 2, \dots, ccnt\} - D$ ) is obtained to judge whether it is less than the preset threshold  $K_t$  (Steps 806 and 807). When  $d_{p,q}$  is less than  $K_t$ , the following procedure takes place. Cluster  $C[ccnt+1]$  is newly generated. A sum set of elements contained in clusters  $C[p]$  and  $C[q]$  is registered into cluster  $C[ccnt+1]$  (Step 808) and the elements are deleted (Step 809). Since  $C[p]$  and  $C[q]$  are no longer necessary,  $p, q$  are registered into  $D$  (Step 810). Then, dissimilarity  $d_{h,ccnt+1}$  between cluster  $C[h]$  ( $h \in \{1, 2, \dots, ccnt\} - D$ ) and cluster  $C[ccnt+1]$  from time  $t$  to time  $t+S$  is obtained (Step 811).

$d_{h,ccnt+1}$  can be obtained by the following calculation,

where when  $n(k)$  is the number of elements in cluster  $C[k]$  and:

(1) when the clustering method is nearest neighbor method,  $\alpha$

$$d_{h,ccnt+1} = \alpha d_{h,p} + \beta d_{h,q} + \gamma d_{p,q} + \delta |d_{h,p} - d_{h,q}| \quad \dots\dots(6)$$

$= 0.5, \beta = 0.5, \gamma = 0$  and  $\delta = -0.5;$

(2) when the clustering method is furthest neighbor method,  $\alpha = 0.5$ ,  $\beta = 0.5$ ,  $\gamma = 0$  and  $\delta = 0.5$ ;

(3) when the clustering method is group average method,  $\alpha = n(p)/n(ccnt+1)$ ,  $\beta = n(q)/n(ccnt+1)$ ,  $\gamma = 0$  and  $\delta = 0$ ;

(4) when the clustering method is centroid point,  $\alpha = n(p)/n(ccnt+1)$ ,  $\beta = n(q)/n(ccnt+1)$ ,  $\gamma = -n(p)n(q)/n(ccnt+1)^2$ , and  $\delta = 0$ ;

(5) when the clustering method is median method,  $\alpha = 0.5$ ,  $\beta = 0.5$ ,  $\gamma = -0.25$  and  $\delta = 0$ ; or

(6) when the clustering method is Ward method,  $\alpha = \{n(h)+n(p)\}/\{n(h)+n(ccnt+1)\}$ ,  $\beta = \{n(h)+n(q)\}/\{n(h)+n(ccnt+1)\}$ ,  $\gamma = -n(h)/\{n(h)+n(ccnt+1)\}$  and  $\delta = 0$ .

Then, "1" is added to the variable  $ccnt$  indicating the number of the generated clusters (Step 812). The above-described procedure is repeated until the minimum value of updated  $d_{i,j}$  ( $i, j \in \{1, 2, \dots, ccnt\}$ -D) exceeds  $K_t$ .

When the minimum value  $d_{p,q}$  of  $d_{i,j}$  exceeds  $K_t$  at Step 807, clustering is finished and the results are output. First, clusters which do not contain a null set are judged and determined among clusters  $C[1]$  to  $C[ccnt]$  and the total number thereof is entered as  $cmax$  (Step 813). Then,  $cmax$  number of clusters  $A[1], \dots, A[cmax]$  are generated (Step 814). Average expression levels of genes contained in the clusters without a null set are calculated, that is, to obtain  $G'_p = (G_{i1} + \dots + G_{ik})/k$  for clusters  $C[p] = \{i_1, \dots, i_k\}$ . Where the obtained values sequentially aligned in a descending order are  $G'_{p1}, \dots, G'_{pcmax}$ ,  $A[1], \dots, A[cmax]$  are registered as  $C[p_1], \dots, C[p_{cmax}]$  (Step 815). Finally, the total cluster number  $cmax$  and clusters  $A[1], \dots, A[cmax]$  are output (Step 816), thereby ending the whole process.

Figure 10 is a flowchart showing detailed algorithm of the displaying process shown in Figure 4. This algorithm reads out array  $l[I]$  and displays expression data of a corresponding gene.

First, let value  $i$  be "0" (Step 1000) and repeat the following procedure for each gene expression data until value  $i$  equals  $lmax$  (Step 1001). Then, the values of expression data  $g[k][x]$  ( $k = 0, 1, \dots, n$ ) corresponding to a single row of a gene indicated by  $x = l[i].index$  is converted to correlating display colors to be displayed as an  $i^{th}$  row (Step 1002). The dividing line between the clusters is drawn immediately below the just displayed  $i^{th}$  row from time  $l[i].linepos$  to  $T_{end}$  (Step 1003).

Where  $l[i].linepos$  is initial value  $T_{end}$ , there is no need of drawing a dividing line between the clusters.  $i$  is incremented for 1 (Step 1004) and the entire procedure is ended when  $i$  becomes  $lmax$  at Step 1001.

By the above-described process, expression patterns of different genes can effectively be displayed such that the patterns are arranged to be the same at the beginning of the clustering-applied region and become different at some time point in the region, as shown in Figure 2.

On the other hand, gene expression patterns of different genes can effectively be displayed such that the patterns are arranged to be different at the beginning of a clustering-applied region and become the same at some time point in the region, as shown in Figure 3. Such display can be realized by setting  $l[i].linepos$  to  $T_{start}$  at Step 609 (Figure 6), setting  $t$  to end at Step 611, setting the judgment condition  $t+S=end$  to  $t-S=start$  at Step 703 (Figure 7), substituting  $t \leftarrow t-1$  for  $t \leftarrow t+1$  at Step 711, and drawing a dividing line between clusters within a range  $T_{start}$  to

l[i].linepos. This means that the end of a slit is set to  $T_{end}$  at the beginning and then clustering step is performed while shifting the slit in a reverse direction along the time axis.

Hereinafter, an exemplary application of such clustering method will be described, where clustering is performed by shifting the slit from the beginning of the clustering-applied region in a forward direction along the time axis to give a display shown in Figure 11. When expression patterns resembling each other (enclosed with dotted lines 1101 and 1102 in Figure 11) are obtained, these genes are marked (1103) and clustering is performed for them from the end of the clustering-applied region 201 in the reverse direction along the time axis. If the marked genes (1103) are at close locations as shown in Figure 12 (e.g., (1) and (4), (3) and (6)), these genes should have different expression patterns at the beginning and should become to have the same expression patterns at some point. Such bi-directional clustering allows easy guessing of expression status of each gene.

Furthermore, where  $T_{start}$ ,  $T_{end}$  and slit width  $S$  are set to  $T_0$ ,  $T_n$  and  $n$ , respectively, the same display as that of the result of P. Brown et al. mentioned in the Background of the Invention can be obtained.

The present example is not limited to the above description, and details can be modified upon practice. For example, the boundary where the expression pattern changes can be displayed with a combination of known display formats, such as a flicker display, a bright display and a color reversion display.

The processing by the clustering processor 105 can be stored as a program in a storage medium (e.g., CD-ROM) to be provided to a user of a computer.

The data of genes is not limited to time sequential expression data, and the horizontal axis (time axis) shown in Figures 2 and 3 may represent other basis. For example, expression patterns of cancerous and normal cells from the same human tissue can be combined and simultaneously clustered so as to find genes that specifically function to cancer, genes that specifically function to normal cells and genes that specifically function to both types of cells.

Figure 23 is a view showing an example of combining and clustering cancerous and normal cells according to the present embodiment. Referring to a gene group 1 (2301) in Figure 23, genes as a whole are strongly expressed in the cancerous cells while some of the genes are expressed but some are not in the normal cells. Referring to other gene group 2 (2302), while weakly expressed genes are gathered in the cancerous cells, some of the genes are expressed but some are not in the normal cells. By simultaneously clustering two types of cells as described above, the behaviors of genes can generally be understood in detail.

Other than comparing difference between states at different time points or between states of tissues, comparison can be made for difference between species such as human and yeast, difference between sites of individual such as stomach, large intestine and heart, difference of expression patterns obtained upon electric shock, shock by high temperature or shock by low temperature, difference between presence and absence of an artificial condition, for example, expression patterns before and after drug administration, or difference of a combination thereof.

Hereinafter, another embodiment of the invention will be described.

Figure 13 shows an exemplary display of results of a clustering analysis according to the present invention.

The display shown in Figure 13 is based on a part of the left part corresponding to the part surrounded by a dotted line 2601 on the right part of Figure 26, the left part showing the entire results of clustering a mass amount of gene expression pattern data. However, Figure 13 may represent the entire results of clustering shown on the left part in Figure 26.

In Figure 13, a dendrogram cross-cutting line 1302 movable on a phase scale bar 1301 can be controlled by a user with a pointing apparatus such as the mouse 104 to find out the expression status at a second section ((2)) of the phase scale bar 1301. It indicates information 1303 (a round number of clusters) that three gene groups exist at the second section as a result of the clustering.

A dividing line 1304 is drawn on the gene expression pattern data 1300 and the resulting gene groups A, B and C are specified (by a gene group-specifying marks 1305).

Figure 14 shows a specific structure of the gene expression pattern data 100 stored in the storage memory 101. The gene expression pattern data 100 exemplified here has m number of vector data 1402 corresponding to the number of gene IDs 1401. The indices of the arrays correspond to n number of experiment cases. Each element of the arrays stores numerated data of gene expression levels (brightness of the hybridized fluorescent signals).

Figure 15 shows exemplary cluster structures utilized upon clustering. There are two types of cluster structures, namely a

cluster structure 1501 whose type member value is "leaf" (left) and a cluster structure 1502 whose type member value is "node" (right).

The leaf-type cluster structure 1501 corresponds to expression pattern data of each gene (i.e., each array data of Figure 14), and the gene ID indicates a value of the gene ID member (e.g., 17). The "level" of the cluster is set to zero.

The node-type cluster structure 1502 is sequentially generated upon the joining process during clustering. The two clusters prior to joining can be reached based on the values of the left member and the right member, and the distance therebetween is stored as a value of the distance member. The level of the cluster is set considering a value of a recognition error range (e.g., 5).

Figure 16 is a diagram showing a structure of data generated during the course of clustering analysis. At the beginning, there are only the leaf-type cluster structures 1501. Then, every time the structures 1501 are paired during the course of clustering analysis, a node-type cluster structure 1502 is generated, thereby configuring a tree-structure. The node-type cluster structures 1502 are linked in the order of generation so that it can be reached from the array `node_clusters[]`. Variable `nclus 1601` gives the total number of the generated node-type cluster structures 1502.

Figure 17 is a diagram showing an example of a data structure for storing the number of gene groups resulting from a certain analysis and display information for drawing the dividing line 1304 between the gene groups. The interval structures 1701 corresponding to the phase of the phase scale bar 1301 are sequentially generated during the grouping process considering the recognition error range, and are linked so that they can be reached from the array

intervals[] 1702. The variable level stores the total number of the generated interval structures.

Each interval structure 1701 has members such as `num_clust`, `min_dist`, `max_dist` and `borders`. The `num_clust` member has information of the number of the gene groups upon grouping, `min_dist` and `max_dist` members have the minimum and maximum values, respectively, in the phase with respect to the distance between the clusters, and the `borders` member has the positional information (the line number) for drawing a dividing line in the gene expression pattern data 1300.

In order to determine which vector data corresponding to the gene ID (Figure 14) should be displayed on which line of the gene expression pattern data 1300, the array `disp_leaf_clusters[]` 1703 is provided with the gene ID value to be displayed as  $i^{\text{th}}$  array element corresponding to  $i^{\text{th}}$  line.

Figure 18 is a flowchart showing a general process according to a method for displaying gene expression patterns of the present embodiment.

First, the gene expression pattern data 100 stored in the storage medium 101 is read by the display processor 105 (Step 1800). The specific structure of the expression pattern data 100 is shown in Figure 14.

Next, each of the parameters necessary for the clustering analysis is set (Step 1801). At this point, the recognition error range entered by the user with the keyboard 103 is accepted and stored as variable `E`. The recognition error range provides a threshold for defining dissimilarity or similarity between expression pattern data of two genes shown in Figure 14. Specifically, when the difference between the expression patterns is

greater than the recognition error range, they are distinguished as distinctive data.

After setting the parameters, clustering analysis is performed (Step 1802). During this clustering analysis, information necessary for the display according to this embodiment is collected and display data is calculated, which will be described later in detail.

Finally, the results of the analysis are displayed (Step 1803). The previously collected and calculated display data are used for the characteristic display of the present embodiment (i.e., the phase scale bar 1301, the dendrogram cross-cutting line 1302, the dividing line 1304 of the gene expression pattern data, and the gene-group-specifying marks 1305).

It is also possible to display the gene-group-specifying marks 1305 only for clusters consisting of at least a predetermined number of gene expression data, and not to display for clusters consisting of data less than the predetermined number of gene expression data.

Figure 19 is a flowchart showing the process of clustering analysis (Step 1802) shown in Figure 18 in detail with respect to the process of generating a cluster tree (the first-stage execution).

Referring to Figure 19, m number of vector data 1402 corresponding to the gene IDs 1401 shown in Figure 14 are registered as m number of leaf-type cluster structures 1501 as target clusters to be joined (Step 1900). Then, cnum (the number of the clusters to be joined) and nclus (the number of the generated node-type cluster structures 1502) are initialized as "m" and "0", respectively (Step 1901). Then, cnum (the number of clusters to be joined) is judged

whether it is equal to "1" or not (Step 1902). If not, the following procedure is repeated until it is equal to "1".

First, two clusters having a minimum relative distance therebetween are selected from the registered cluster structures (Step 1903). A node-type cluster structure C is newly generated (Step 1904), and the number of the node-type clusters is incremented (Step 1905). The new node-type cluster structure is registered as the `nclusth` structure of the array `node_clusters[]` (Step 1906). The two clusters selected in Step 1903 are registered as the left and right members of the new node-type cluster structure, and the distance therebetween is registered as the `distance` member of the new node-type cluster structure (Step 1907).

It is also possible to set up a judging basis for which one of the two clusters should be the left or right member.

Finally, the two cluster structures are removed from the target cluster structures to be joined, a new node-type cluster structure is registered (Step 1908) and the value of `cnum` (the number of the clusters to be joined) is decremented (Step 1909).

When the value of `cnum` equals "1" upon the judgement of Step 1902, the process proceeds to a procedure of the flowchart shown in Figure 20 (process of setting the cluster levels).

Figure 20 is a flowchart for setting the cluster level (the second-stage execution).

The node-type cluster structures generated by the first-stage process are all registered in the array `node_clusters[]` (Figure 16) and the values of their `distance` members are generally aligned in an ascending order according to the index of the array. However, depending on the selection of algorithm, the `distance` members may not be aligned in an ascending order. Thus, first, the `distance`

member of each node-type cluster structure registered in the array `node_clusters[]` is verified (Step 2000).

When the distance members are judged that they are not aligned in an ascending order by this verification, `node_clusters[]` is subjected to a sorting process. Alternatively, only the part not in an ascending order may be detected and subjected to an additional process.

Then, each variable is initialized (Step 2001). Specifically, the variables are counter `i` of the array `node_clusters[]` (initial value: 1), cluster level `level` (initial value: 1), a value of distance member `curr_dist` of the presently processed node-type cluster (initial value: 0), and a value of distance member `prev_dist` of the previously processed node-type cluster (initial value: 0).

The value of counter `i` and the value of variable `nclus` are compared to perform the following procedure to each element of the array `node_clusters[]` (Step 2002).

First, a distance member value of an  $i^{\text{th}}$  node-type cluster is stored as variable `curr_dist` (Step 2003) to judge whether or not the difference between the variables `curr_dist` and `prev_dist` is smaller than the recognition error range `E` determined by the user (Step 2004).

When the difference is greater than `E`, the variable `level` is incremented (Step 2005) and an interval structure is newly generated. Then, the values of `prev_dist`, `curr_dist` and `(nclus-i+1)` are set as `min_dist`, `max_dist` and `num_clust` members of the newly generated structure, respectively, and register the newly generated interval structure as a  $\text{level}^{\text{th}}$  element of the array `intervals[]` (Step 2006). The `borders` member is provided with a null set as a default value.

When the difference between values `curr_dist` and `prev_dist` is less than `E` in Step 2004, steps 2005 and 2006 are omitted and the following procedure proceeds.

The value of the variable `level` is registered as a value of the `level` member of the node-type cluster structure registered in the `node_clusters[i]` (Step 2007).

Finally, the `curr_dist` value is shifted to `prev_dist`, the counter `i` is incremented (Step 2008) and the process returns to Step 2002.

When all of the elements of the array `node_clusters[]` are completely subjected to the procedure of steps 2003 to 2008 (Step 2002), a procedure shown in Figure 21 proceeds.

Figure 21 is a flowchart showing the process of clustering analysis (Step 1802) shown in Figure 18 in detail with respect to generating display data (the third-stage execution).

First, variable `j` indicating an index of array `disp_leaf_clusters[]` is set to "1", and a value of an argument `cluster` used for the subsequent display data generating process (Process A) is set to a node-type cluster structure indicated by the `nclusth` element of array `node_clusters[]` (Step 2100).

Then, Process A (display data generating routine) is called using `cluster` as an argument (Step 2101). In step 2101, Process A is recursively called to collect and calculate display data during the course of tree-walking along the tree-structure of clusters generated according to the flowchart of Figure 19. When this recursive process is finished, the clustering analysis is ended.

Figure 22 is a flowchart showing Process A (display data generating routine) (Step 2101) shown in Figure 21 in detail.



Thus, clustering analysis can be displayed as shown in Figure 13.

First, the gene expression pattern data 1300 can be displayed line by line from the top by utilizing information of array `disp_leaf_cluster[]`. The dendrogram can be displayed based on the tree-structure data having clusters as roots indicated by `node_clusters[nclus]`.

The phase scale bar 1301 and the dendrogram cross-cutting line 1302 which are characteristic of the present embodiment can be displayed with reference to the `min_dist` and `max_dist` member values of each interval registered in array `intervals[]`. The number of the resulting gene groups 1303 can be displayed with reference to `num_clust` member value.

The dividing line 1304 between the displayed gene groups in the gene expression pattern data and the gene-group-specifying marks 1305 can be displayed with reference to the `borders` member value.

The processes shown in Figures 18 to 22 can be stored in a storage medium (e.g., CD-ROM) as gene expression pattern analyzing/displaying program to be provided to users via a personal computer, a work station or the like.

Herein, the result of the analysis is displayed on a display device. However, the results can be printed out with a multicolor printer which has recently been greatly improved. According to the present invention, the idea of display also comprises a printed out display.

According to the present invention, expression of a part of cell cycle can be designated to perform clustering in that region in a small range. Based on the displayed results, the user can observe the course of expression status of genes in more detail to







while changing the reference value, and displaying the results according to a predetermined display format.

12. A method for visually displaying expression patterns of multiple genes, comprising a step of displaying a round number of the cluster groups for each clustering phase taking a recognition error range in consideration, for the results of the clustering analysis of the gene expression pattern data.

13. A method for displaying expression patterns of multiple genes according to claim 12, wherein the results of the clustering analysis are displayed such that only clusters including a predetermined or greater number of the gene expression pattern data are displayed as a gene group.

14. An apparatus for displaying expression patterns of multiple genes, comprising:

a storage medium for storing expression pattern data of the genes;

an analysis processor for reading out the stored pattern data of genes for clustering analysis; and

a display processor for displaying a round number of the cluster groups for each clustering phase on a display screen taking a recognition error range in consideration, for the results of the analysis.

15. A storage medium for storing an operational program for visually displaying expression patterns of multiple genes on a display screen, wherein the stored operational program comprises a step of displaying the round number of the cluster groups for each clustering phase taking a recognition error range in consideration, for the results of the clustering analysis of the gene expression pattern data.

## ABSTRACT

The present invention aims at finding out and effectively displaying that different genes give the same expression for having the same function at one time point but give different expressions at another time point for having different roles. Furthermore, the present invention aims at automatically extracting brief groups of clusters from the clustering results of gene expression pattern data so that a user can select a desirable level of grouping.

The present invention comprises the steps of: designating a time segment in the time sequential expression pattern data of multiple genes; and clustering the time sequential expression pattern data within the designated time segment based on a predetermined reference value, repeating clustering within the same cluster in a forward or reverse direction along the time axis while changing the reference value, and displaying the results according to a predetermined display format. The present invention also comprises a step of displaying a round number of the cluster groups for each clustering phase, taking a recognition error range in consideration, from the results of the clustering analysis of the gene expression pattern data. The results of clustering analysis are displayed such that only gene groups consisting of at least a predetermined number of gene expression pattern data are displayed.

FIG. 1

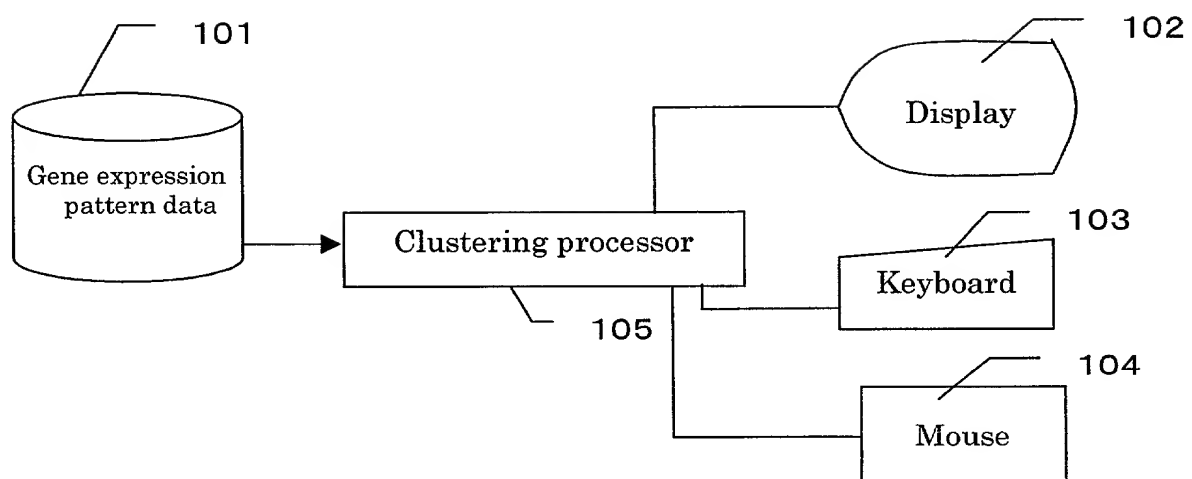


FIG. 2

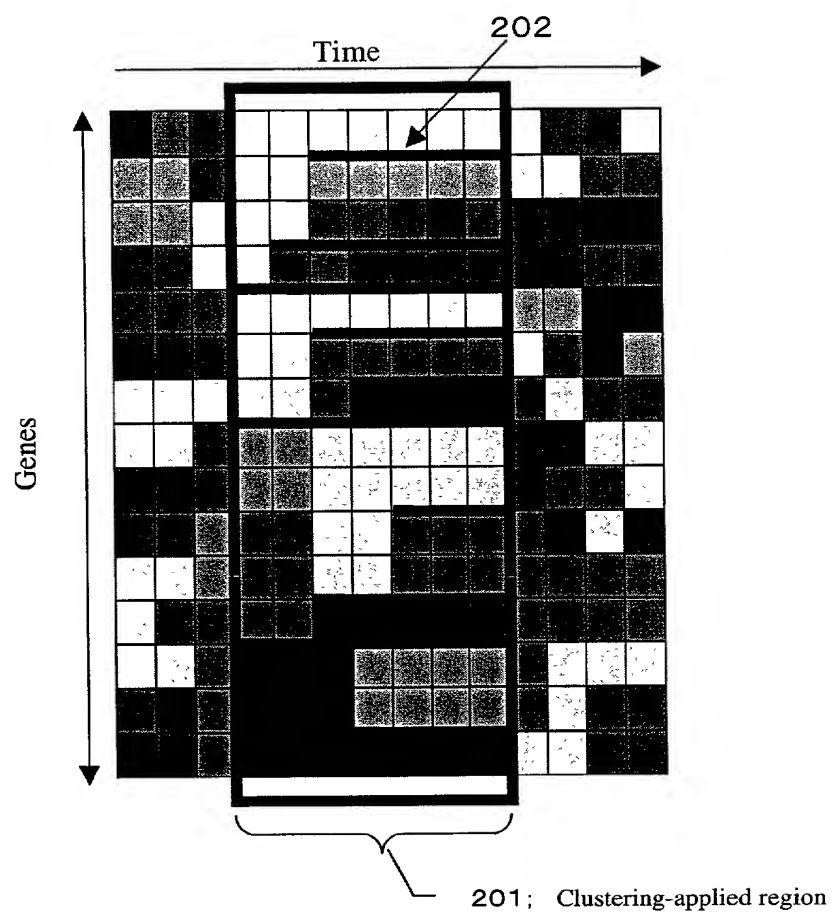


FIG.3

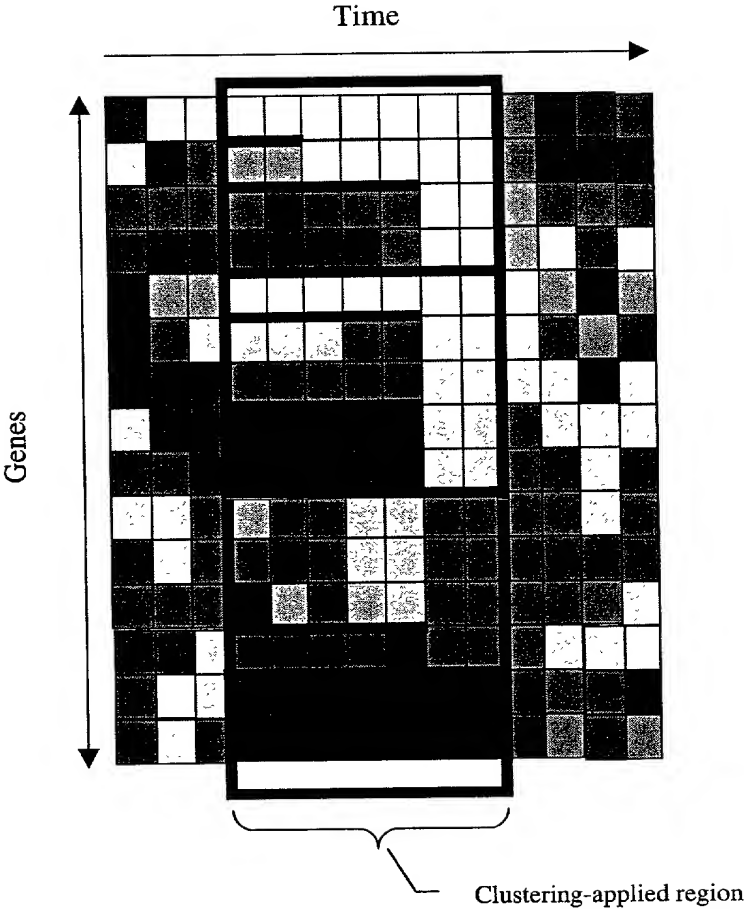


FIG. 4

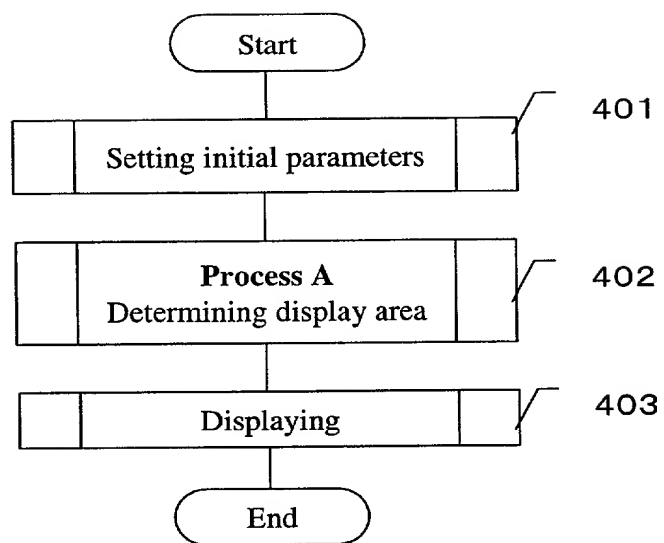


FIG. 5

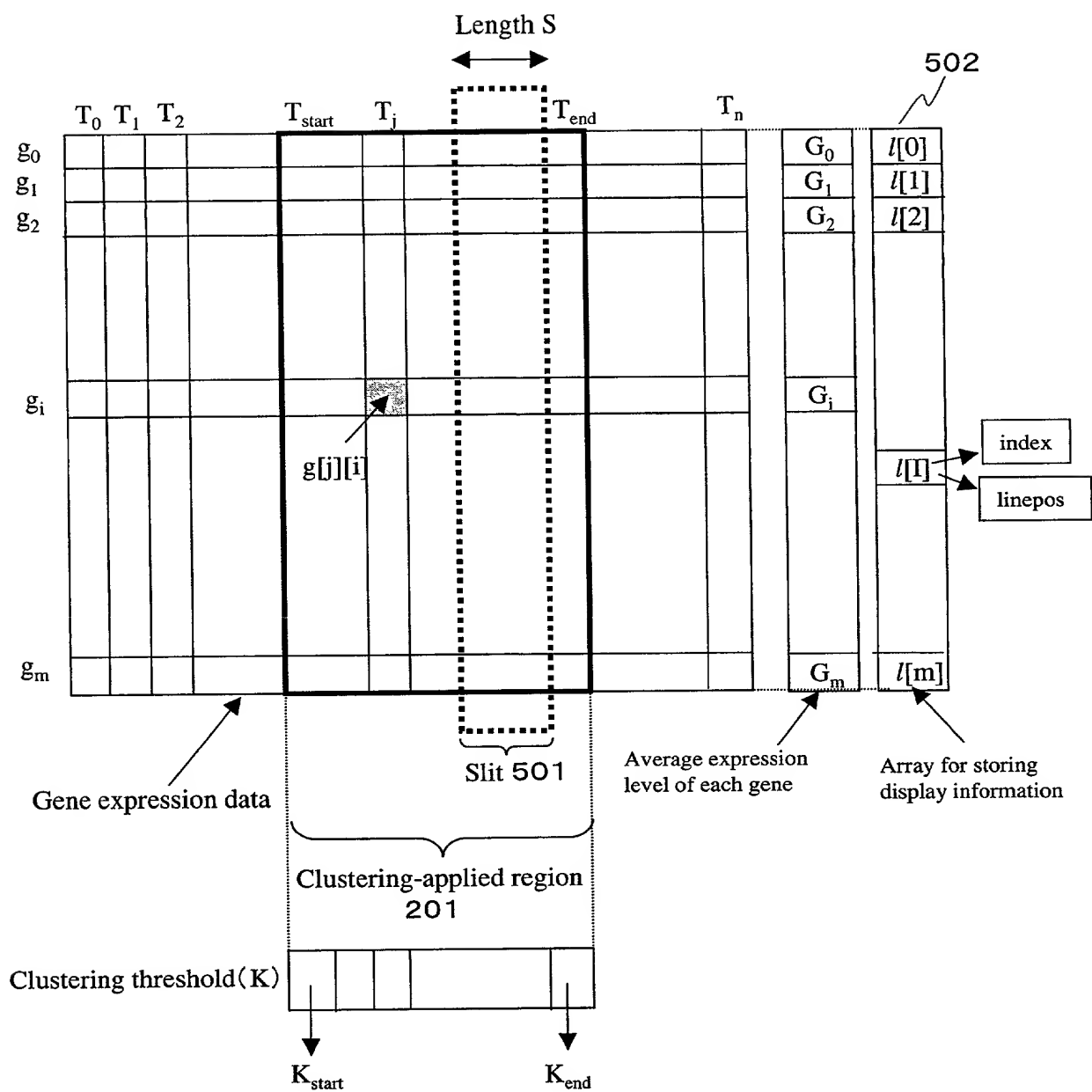


FIG. 6

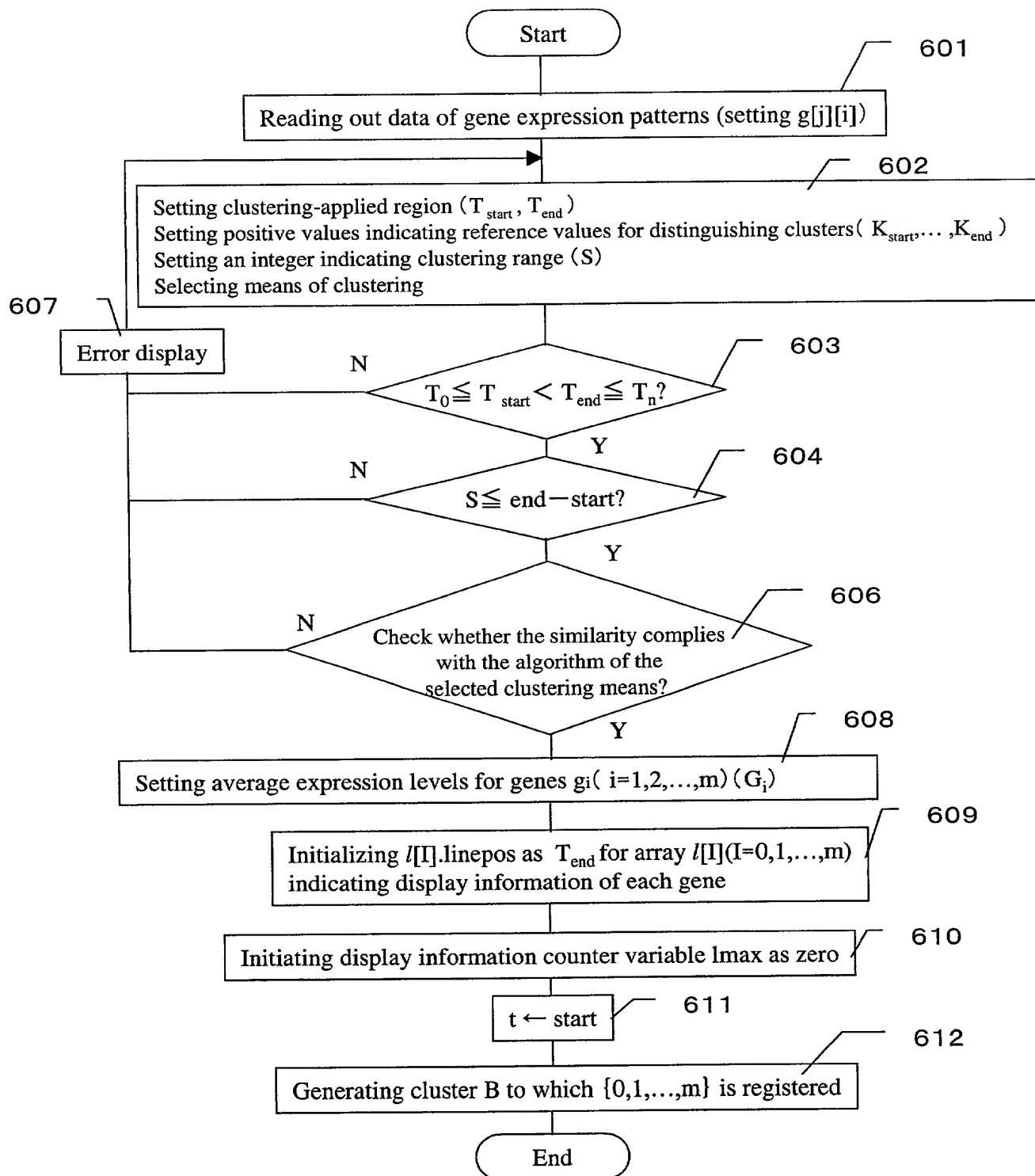


FIG. 7

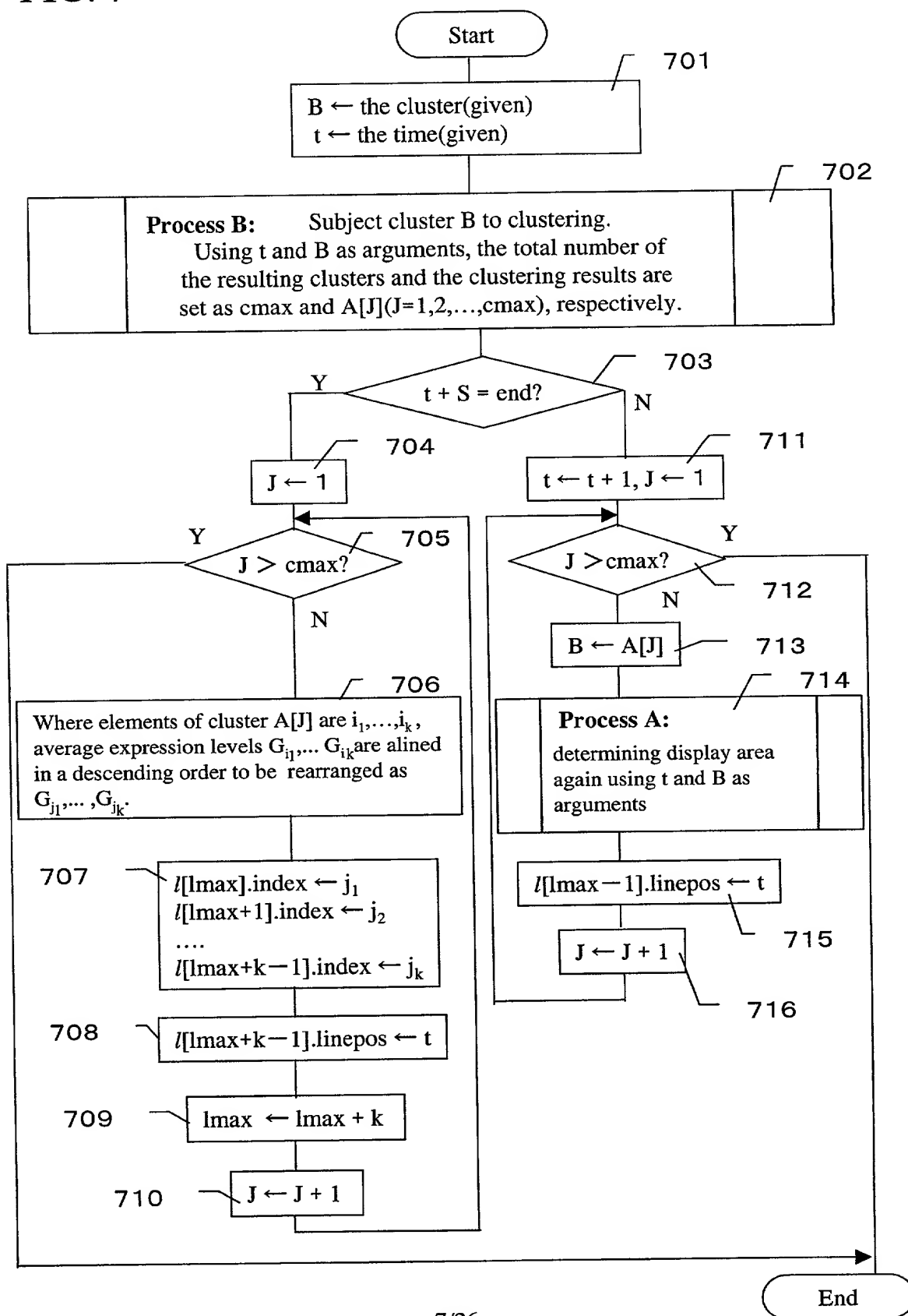


FIG. 8

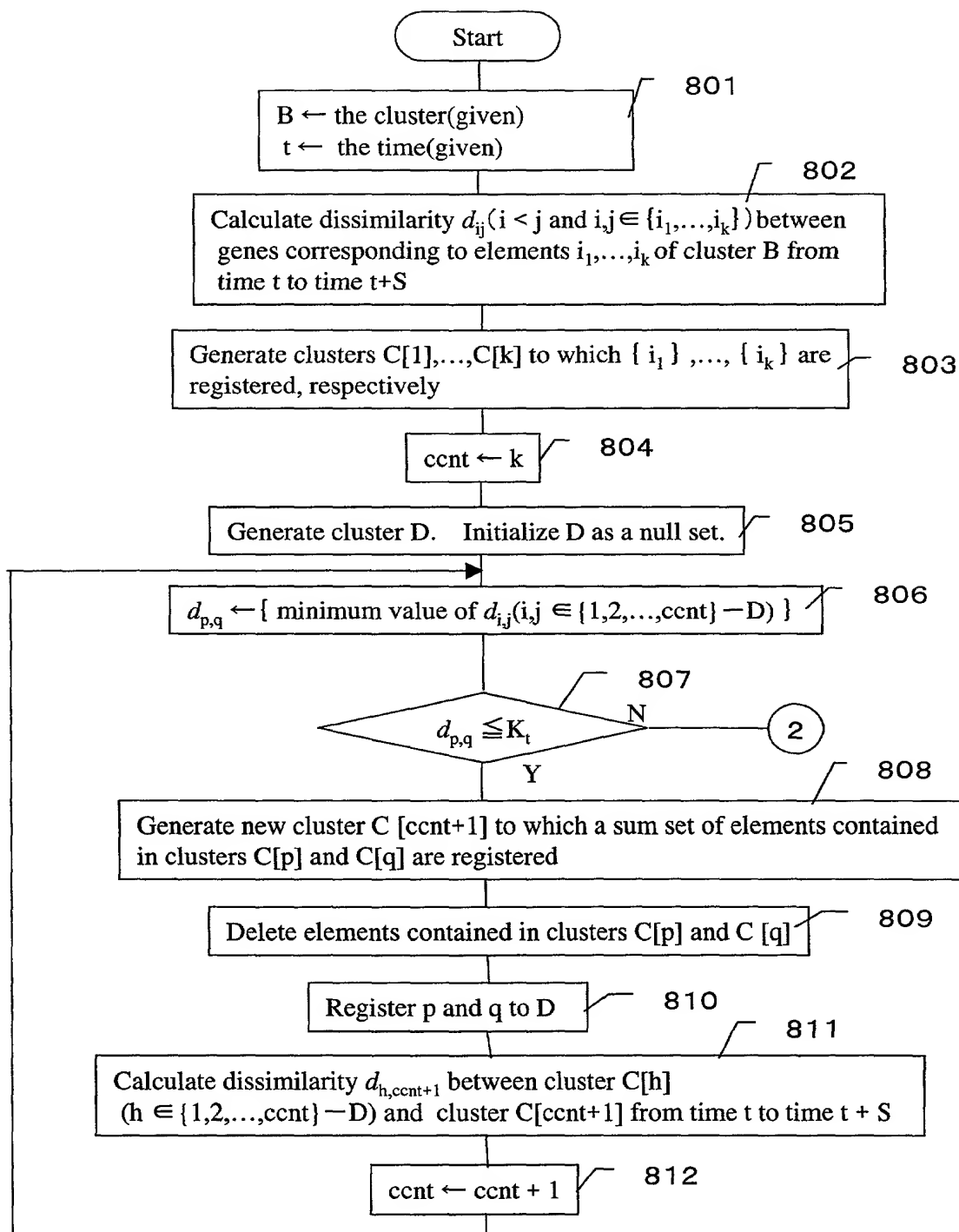


FIG. 9

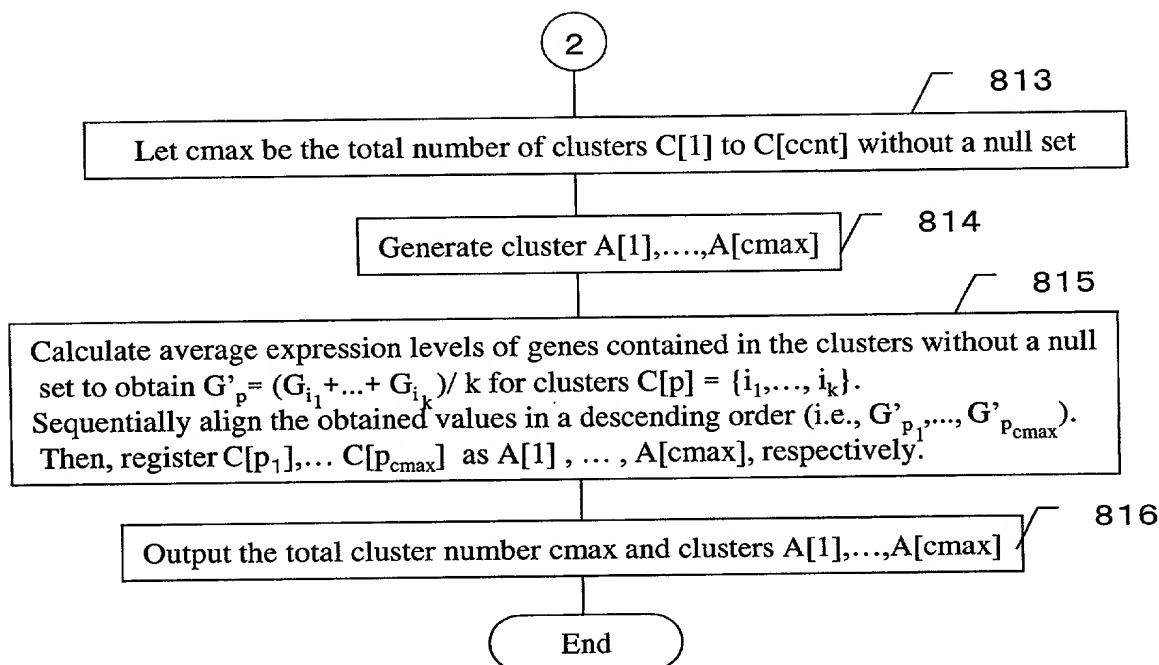


FIG. 10

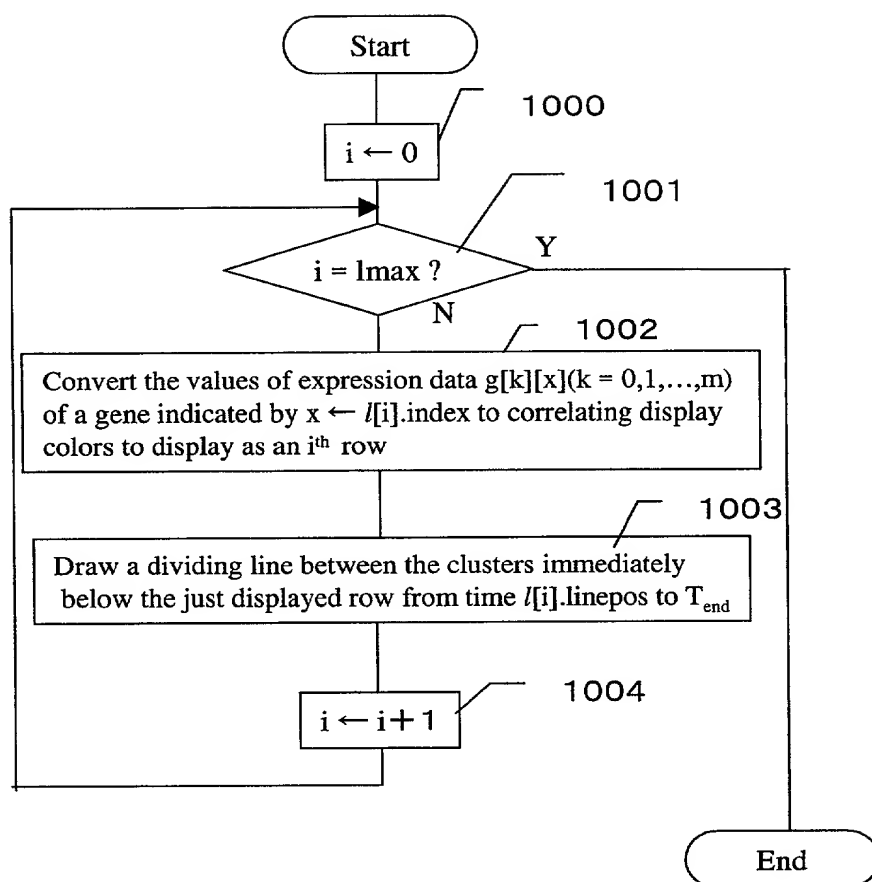


FIG.11

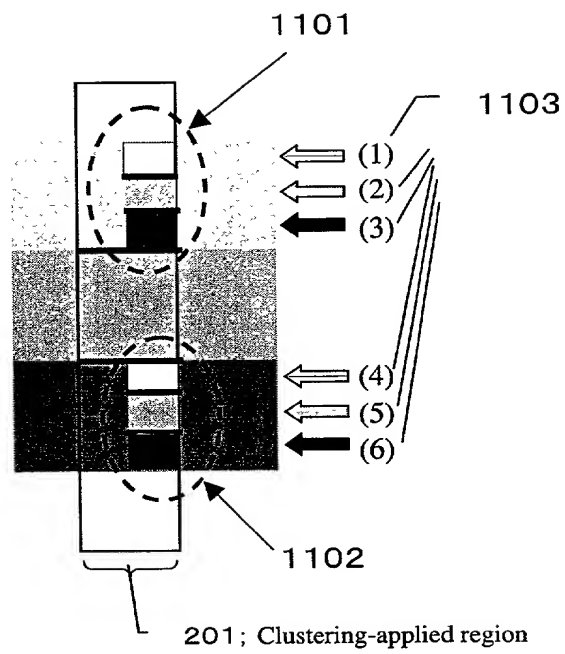




FIG.13

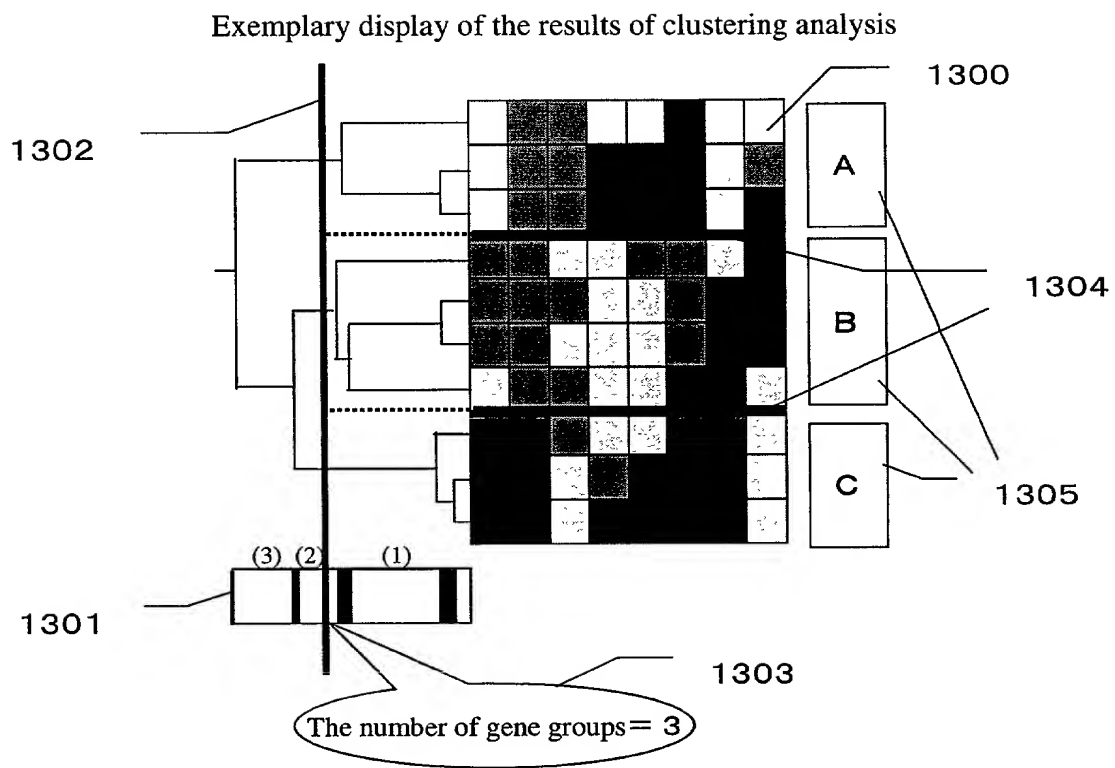


FIG.14

Example of gene expression pattern data

Gene ID (geneID)	Experiment cases									
	1	2	3	4	5	6	..	..	..	n
1	0	1	2	0	3	2	0			
2	1	2	0	0	2	2	1			
⋮										
⋮										
m	0	4	3	6	5	4	0			

1401

1402

m number of vector data

FIG.15

Example of cluster structures

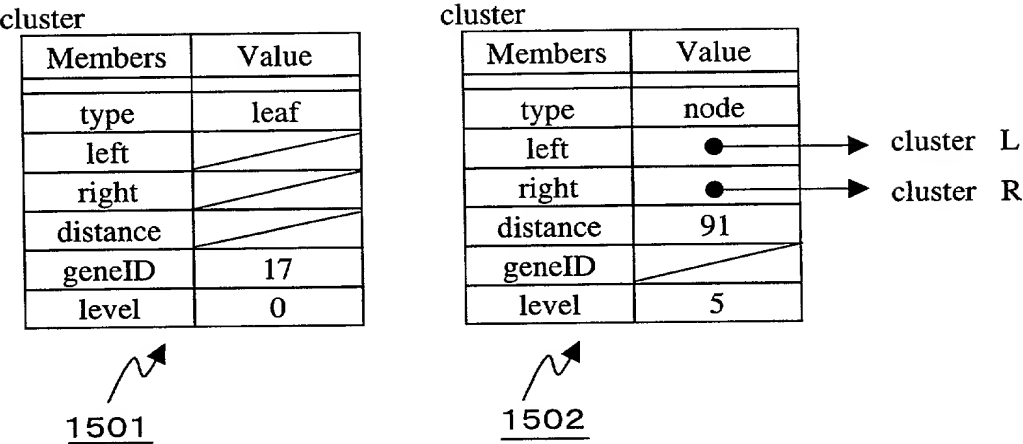


FIG.16

Exemplary structure of cluster tree

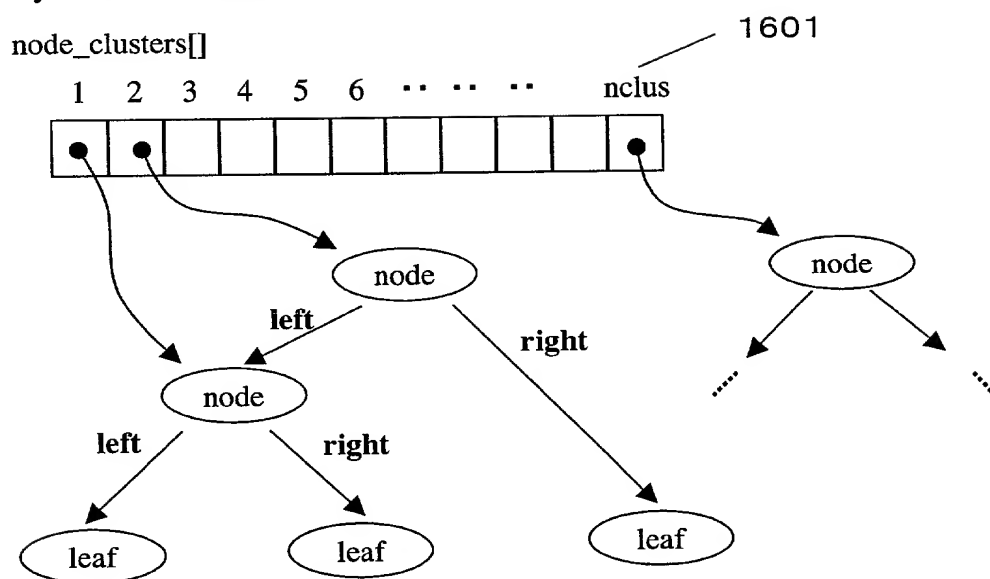


FIG.17

Exemplary display data

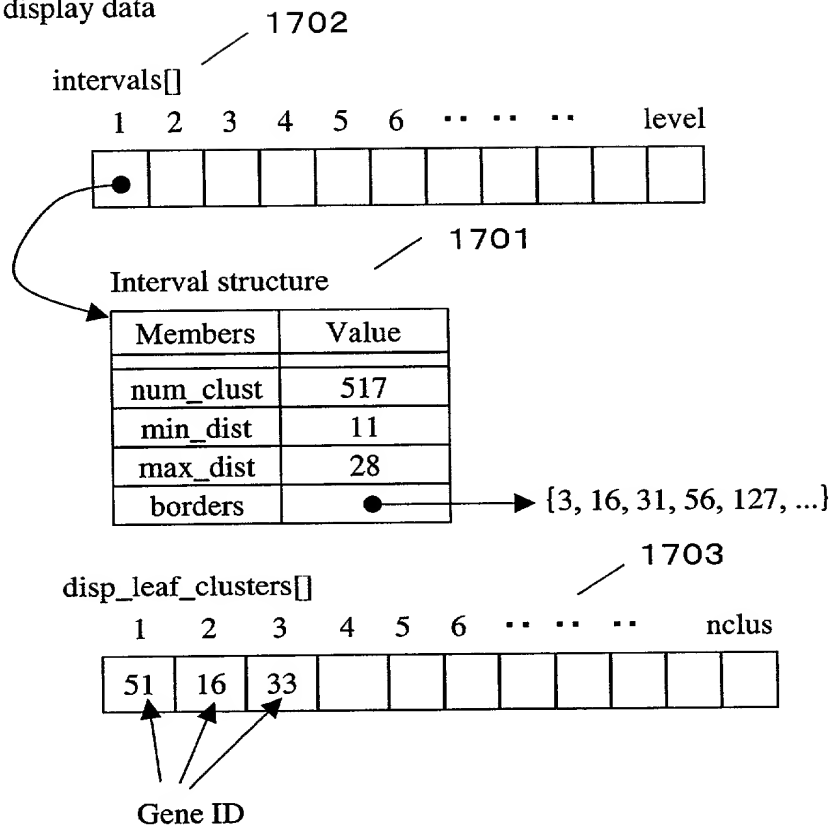


FIG.18

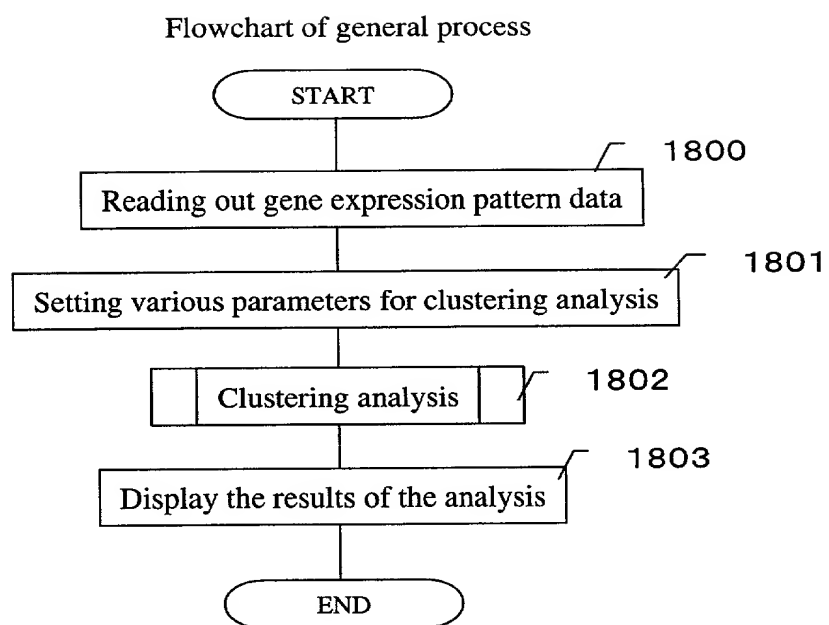
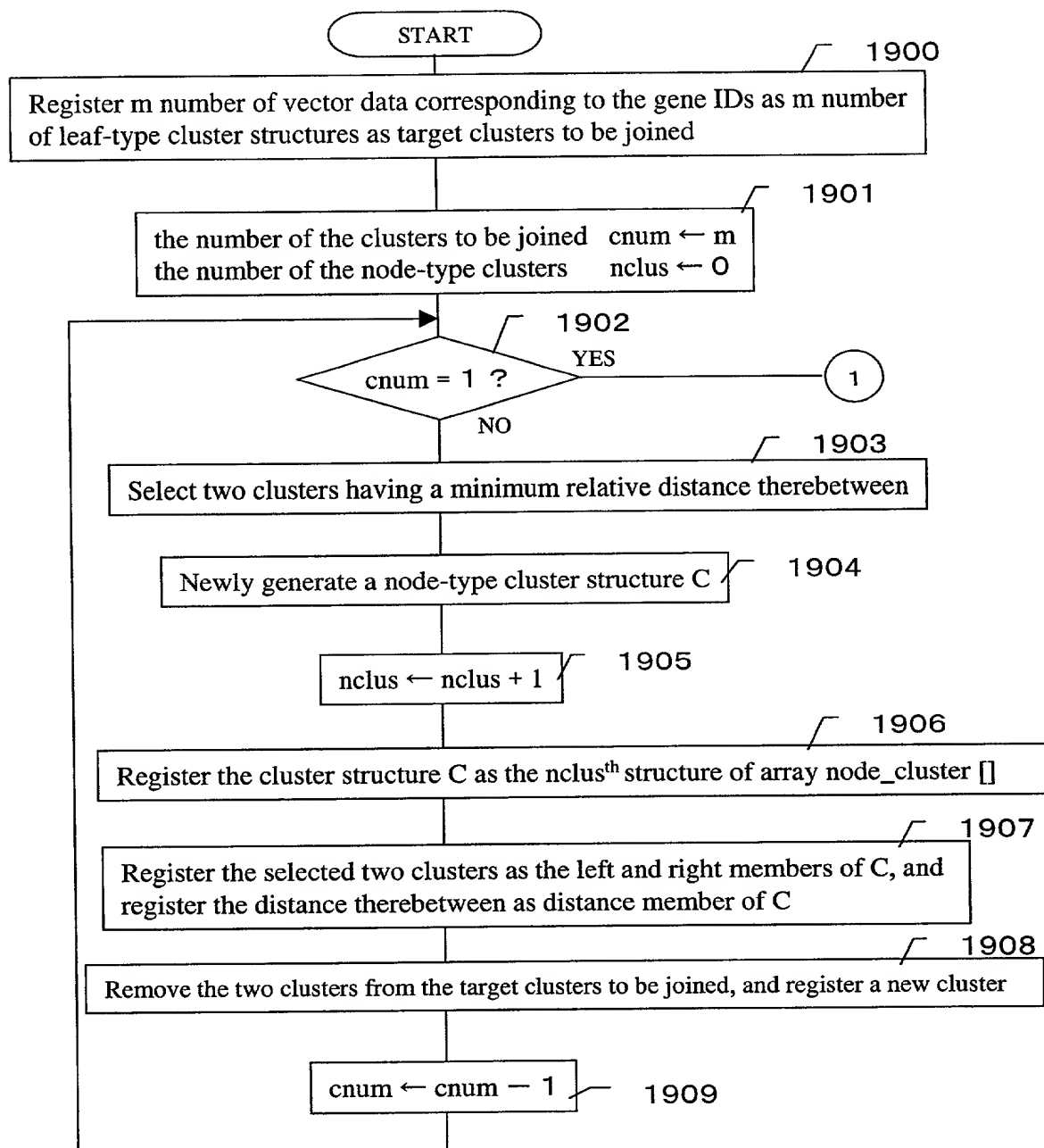


FIG.19

Detailed flowchart of clustering analysis (1: Generating cluster tree)



# FIG.20

Detailed flowchart of clustering analysis (2: Setting cluster levels)

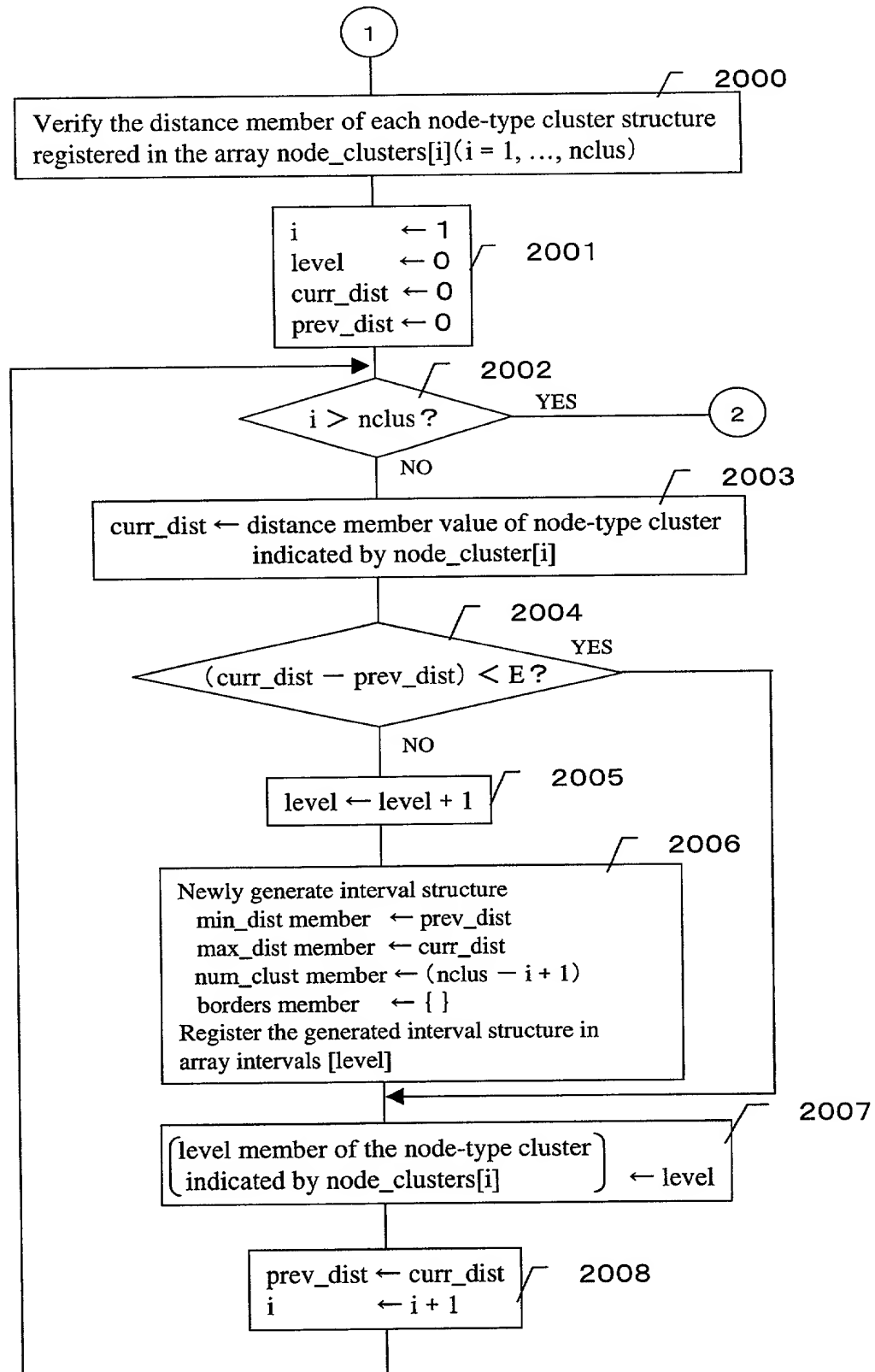


FIG.21

Detailed flowchart of clustering analysis (3: Generating display data)

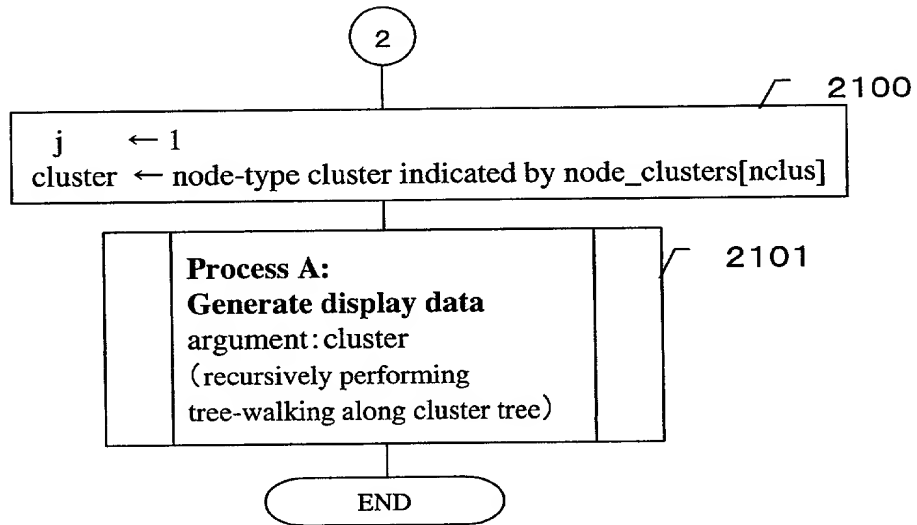


FIG.22

Flowchart of generating display data (Process A)

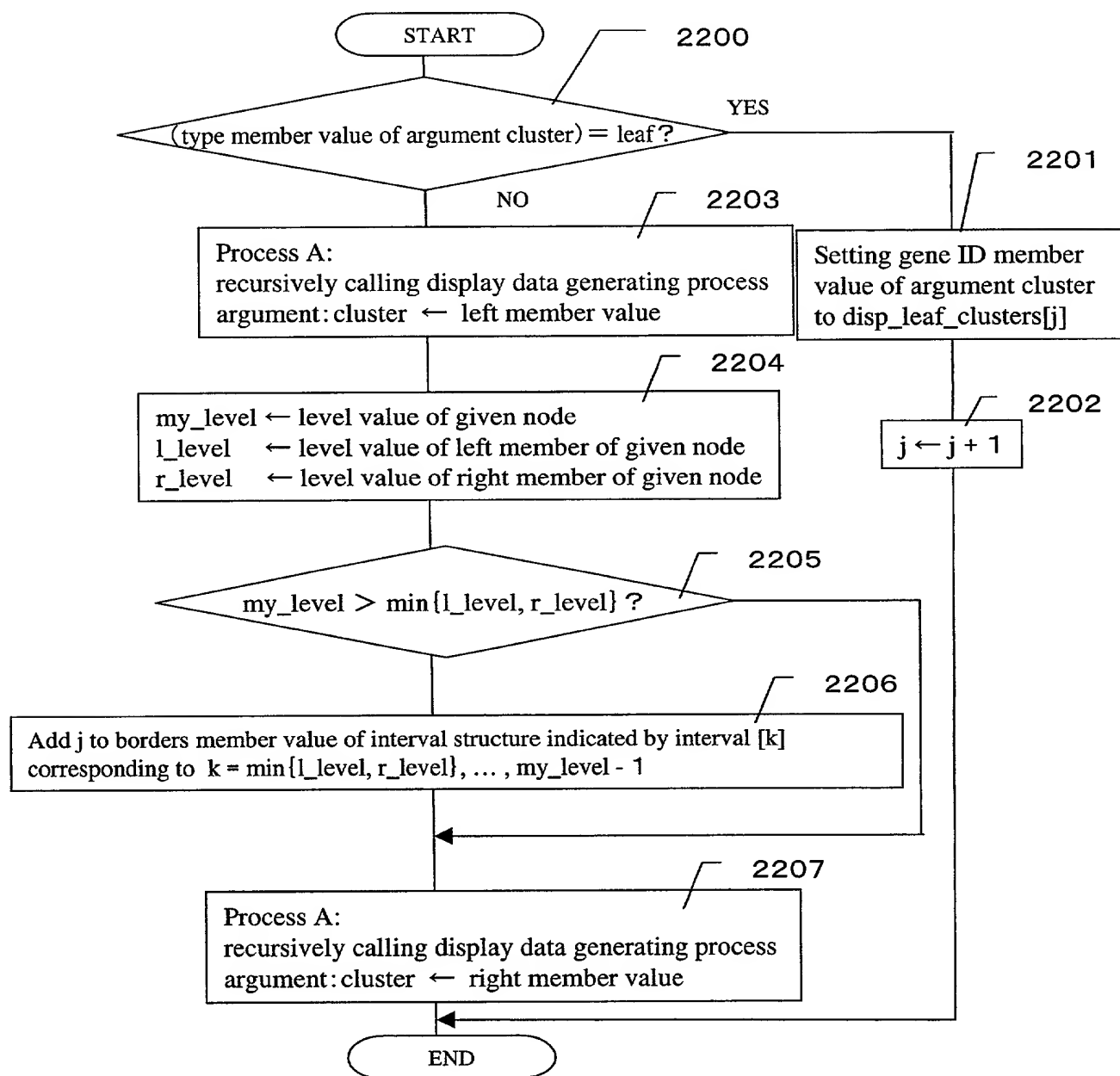


FIG.23

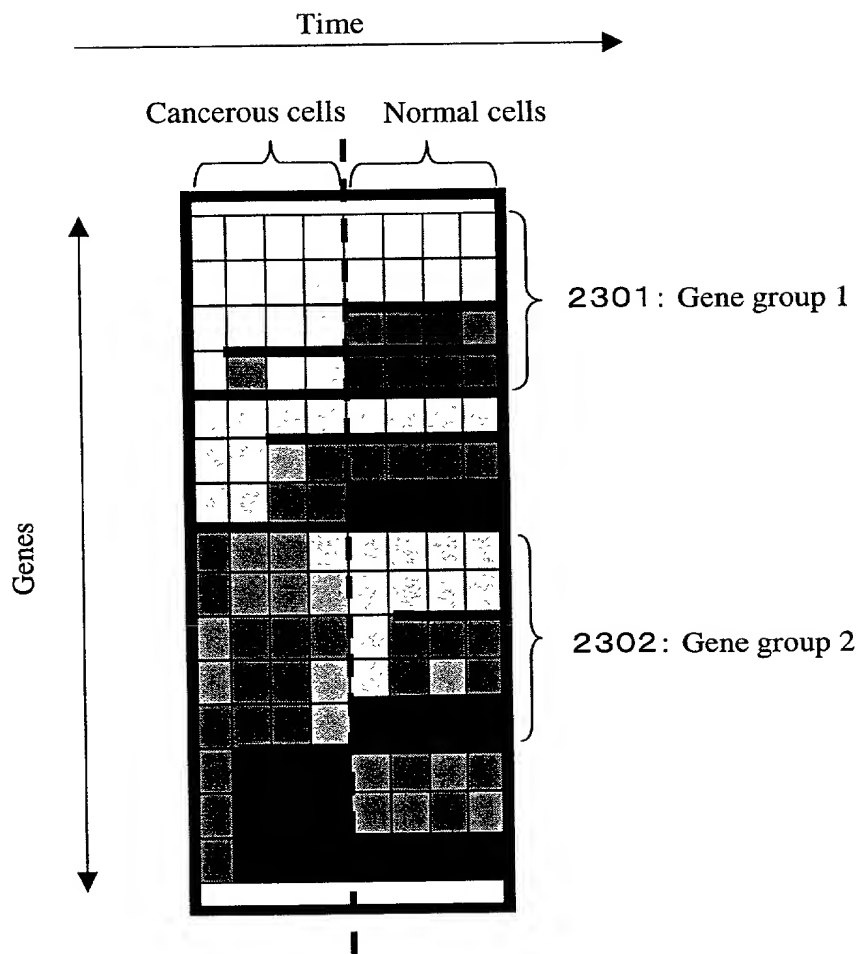


FIG.24

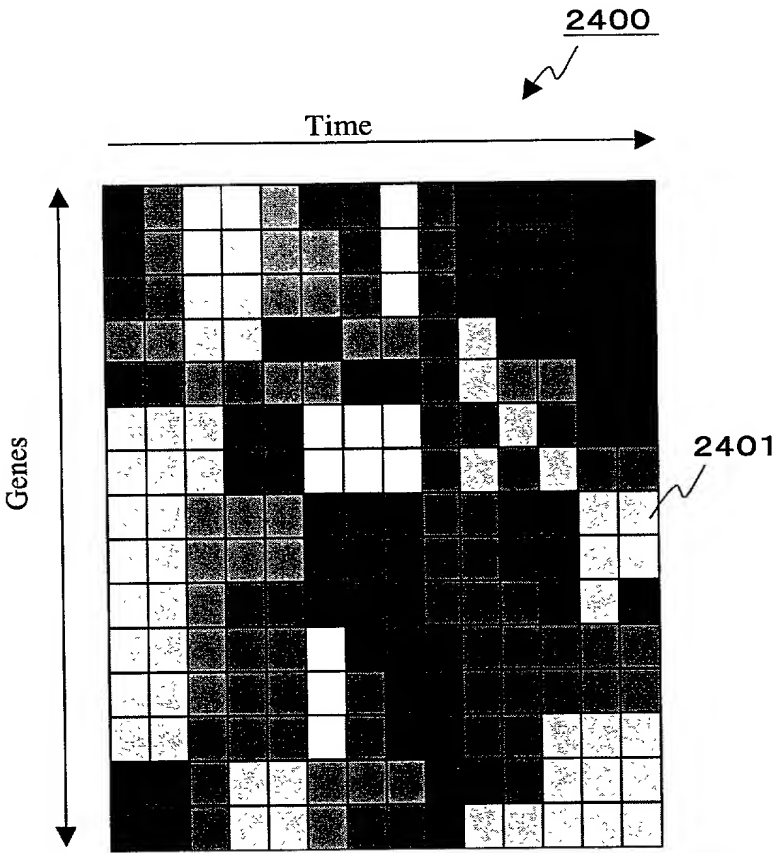


FIG.25

Exemplary display of standard clustering analysis

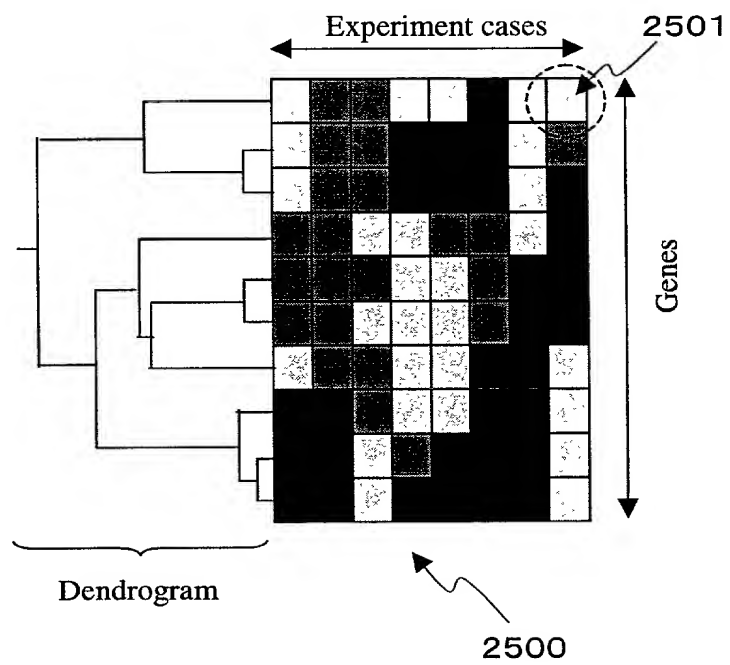
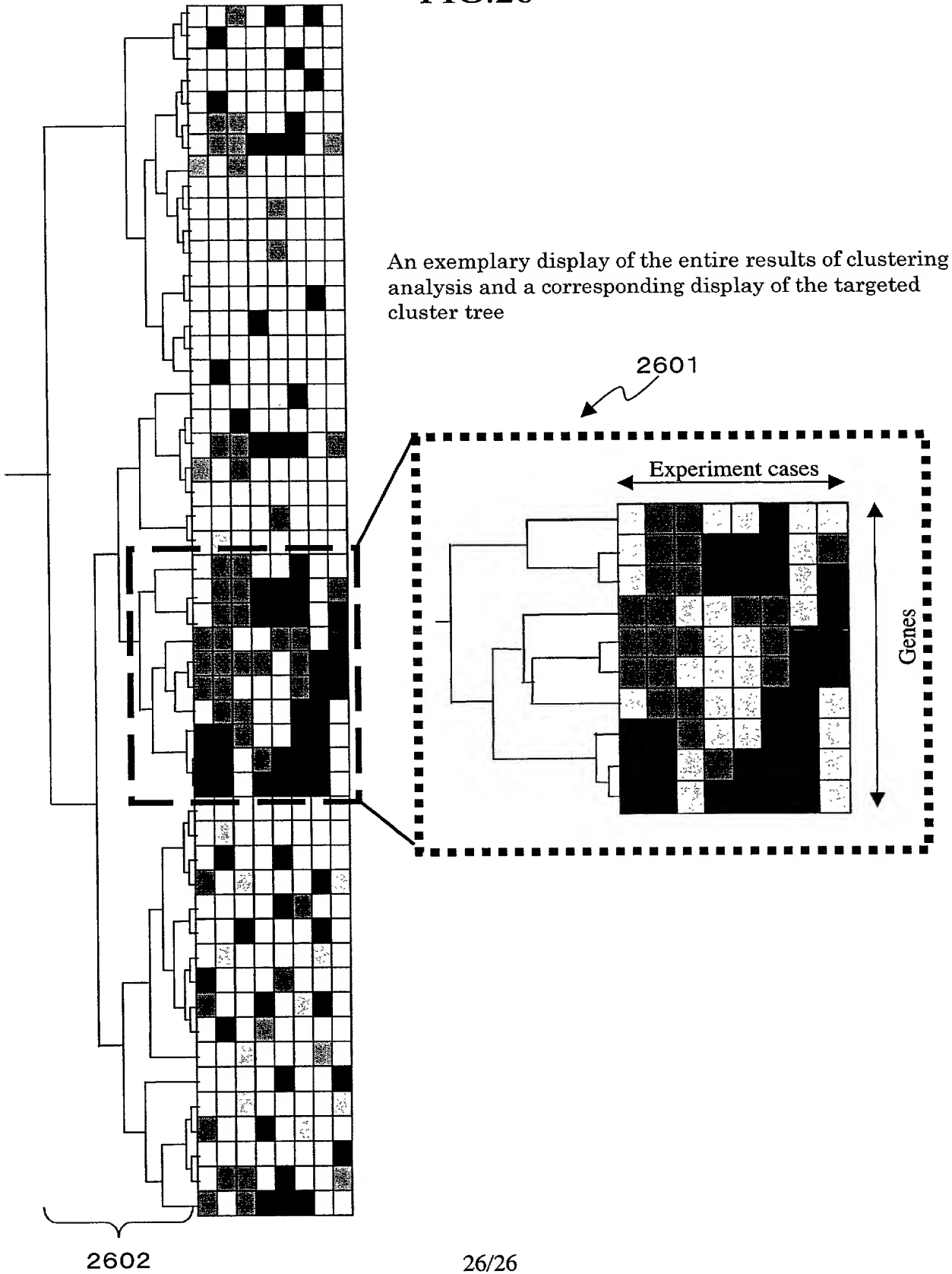


FIG.26



Attorney's Docket No.: 07898-059001**DECLARATION, POWER OF ATTORNEY AND PETITION**

I (We), the undersigned inventor(s), hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I (We) believe that I am (we are) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD AND APPARATUS FOR DISPLAYING GENE EXPRESSION  
PATTERNS

the specification of which

☒ is attached hereto.

☐ was filed on \_\_\_\_\_ as

Application Serial No. \_\_\_\_\_

and amended on \_\_\_\_\_.

☐ was filed as PCT international application

Number \_\_\_\_\_

on \_\_\_\_\_,

and was amended under PCT Article 19

on \_\_\_\_\_ (if applicable).

I (We) hereby state that I (We) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; that I (We) do not know and do not believe that this invention was ever known or used before my invention or discovery thereof, or patented or described in any printed publication in any country before my invention or discovery thereof, or more than one year prior to this application, or in public use or on sale in the United States for more than one year prior to this application; that this invention or discovery has not been patented or made the subject of an inventor's certificate in any country foreign to the United States on an application filed by me or my legal representatives or assigns more than twelve months before this application.

006260-2402960

I (We) hereby claim foreign priority benefits under Section 119(a)-(d) of Title 35 United States Code, of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

I hereby claim the benefit under Section 119(e) of Title 35 United States Code,  
of any United States application(s) listed below.

\_\_\_\_\_  
(Application Number) (Filing Date)

I (We) hereby claim the benefit under Section 120 of Title 35 United States Code, of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Section 112 of Title 35 United States Code, I (We) acknowledge the duty to disclose material information as defined in Section 1.56(a) of Title 37 Code of Federal Regulations, which occurred between the filing date of the prior application and national or PCT international filing date of this application:

## Japan

September 12, 2000

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Date

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Tsunehiko WATANABE  
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